REMARKS

Claims 1 and 15 have been amended to specify the step of injecting spermatogonial cells or testicular cells into the most upper portion of the seminiferous tubule of the recipient.

The avian chimera in claims 1, 13 and 14 has also been amended to refer to "avian germline chimera."

Claims 8 and 9 have been canceled.

Claim 14 has been amended not to refer to canceled claims 8 and 9.

Support for the amendments to claims 1, 13, 14, and 15 can be found throughout the specification, in particular, at page 11, lines 18 to 25. Accordingly, no new matter has been added by the amendments.

The preceding amendments and the following remarks are believed to be fully responsive to the outstanding Office Action and are believed to place the application in condition for allowance.

The Examiner is respectfully requested to reconsider and withdraw the rejections in view of the amendments and remarks as set forth herein.

Rejection under 35 U.S.C. § 112, first paragraph

The Examiner rejects claim 14 as having insufficient antecedent basis for the limitation "the avian." Applicants respectfully traverse the rejection.

Claim 14 refers to and depends from claim 13, which is drawn to an avian chimera. Therefore, claim 14 has sufficient antecedent basis for the limitation of "the avian."

Accordingly, Applicants request that this rejection be withdrawn.

Rejection under 35 U.S.C.§ 102(b)

The Examiner rejects claims 1, 3-6, 10, and 12-15 as being anticipated by Lin (US 2002/0076797, published June 20, 2002). Applicants respectfully traverse this rejection.

The Examiner states that Lin teaches that primitive cells were collected from testes. Applicants disagree with the Examiner's interpretation. There is no explicit description of collecting primitive cells from testis in the Lin publication.

At page 13, paragraph [0120] of this publication, it is stated that "a feeder matrix can be derived from or provided by the organ or tissue in which the primitive cells are located, e.g., the gonad." However, it is noteworthy that this description explains that the feeder matrix or cells, not the primitive cells, can be obtained from the organ of gonad. Thus, there is no evident description that spermatogonial cells or testicular cells are collected from the donor's testis.

At page 21, paragraph [0209] of the Lin publication, there is no specific description as to how transgene-carrying cells were made. In particular, the method of preparing the isolated spermatogonial cells and transfection conditions in the re-injection procedure are not sufficiently elucidated. The description in paragraph [0209] is made just from his assumption or prediction, as it is not supported by any substantial experimental data. When Lin's patent application was published in 2002, as evident from the publication, he just had the knowledge of spermatogonial cell, spermatogonial stem cell, or germline stem cells in the testis theoretically, not with experimental results, because avian spermatogonial cell or germline stem cell culture systems have first been developed by the present inventors, and the transfection conditions of exogenous genes into avian spermatogonial cells had not been established until the present invention.

Furthermore, in the amendments of claims 1 and 15, the limitations described in present

claims 8 and 9, which have not been rejected as being anticipated by the Lin publication, have been incorporated into claims 1 and 15. Therefore, Applicants submit that the rejection under 35 U.S.C. § 102(b) has been overcome.

Accordingly, Applicants respectfully request that this rejection be withdrawn.

Rejection under 35 U.S.C. § 103(a)

The Examiner rejects claims 1-15 as being unpatentable over Lin (US 2002/0076797, published June 20, 2002), in view of Rapp et al. (US 2003/0126629, published July 3, 2003) and further in view of Li et al. (Poultry Science; 81:1360-1364, 2002). Applicants respectfully traverse this rejection.

The present invention, as specified in amended claims 1 and 15, is characterized by the cultured spermatogonial cell population or testicular cell population being injected into the most upper portion of the seminiferous tubule of a recipient. The step of injecting spermatogonial cells or testicular cells into the recipient is significantly important for the production of avian chimeras.

The Examiner states that Rapp et al. also teach an embodiment that "employs injection of the gene delivery mixture, preferably into the seminiferous tubules or into the pete testis, and most preferably into the vas efferens or vas efferentia" (page 15, paragraph 0154). However, attention must be paid to the fact that this description relates to the injection of gene delivery mixture, for example, a nucleic acid, by in vivo methods (e.g., by in vivo transfection or transduction), not to the injection of spermatogonial cells or testicular cells.

Please see paragraphs from [0151] to [0156] (especially [0152]) of the publication of Rapp

et al., in which the inventors classify the method of the sperm-mediated integration of heterologous transgene into two distinct types of method, i.e. *in vivo* method and *in vitro* method.

[0152]

> The first method of incorporating heterologous genetic material into the genome of an avian delivers a nucleic acid using known gene delievery systems to male germ cells in situ in the testis of the male avian (e.g., by in vivo transfection or transduction). The second, in vitro, method of incorporating heterologous genetic materials into the genome of an avian involves isolating male germ cells ex corpora, delivering a polynucleotide thereto and then returning the transfected cells to the testes of a recipient male bird.

According to the specific explanations on pages 15 and 16 of Rapp et al., the *in vivo* method employs genetic materials (not germline cells) for injection, and the *in vitro* method utilizes isolated and genetically modified germ cells for injection. In the *in vivo* method, the gene delivery mixture including nucleic acids, which are described to be injected into the seminiferous tubules of the recipient. However, in the *in vitro* method (germline cell injection method), there is no description that the isolated and genetically modified germ cells are injected into the most upper portion of the seminiferous tubules of the recipient avian. Please note that the method of transplantation of spermatogonial cells or testicular cells was firstly established by the present inventors and published in 2006 (Lee et al., Biol. Reprod. 75:380-386, 2006; Jung et al., Biol. Reprod. 76:173-182, 2007; copies are enclosed). It is well known in the art that delivery of genetic materials via cell transplantation is extraordinarily difficult to accomplish as compared with direct incorporation of genetic materials into the recipient cell or tissue, because cell transplantation essentially requires many techniques such as in vitro culture systems, transfection methods, surgical transplantation, and theoretical background knowledge of developmental stage

of animals.

Thus, it is evidently clear that Rapp et al. do not disclose the technical feature of the currently amended claim 1, which requires injection of spermatogonial cells or testicular cells into the most upper portion of seminiferous tubules of the recipient.

In addition, paragraphs [0151]-[0156] refer to "sperm-mediated integration of heterologous transgenes." Please note that in the art "sperm-mediated" is understood to be only spermatozoa mediated methods (Nature Biotechnology 17:636, 1999; Theriogenology 57:189-201, 2002; Reproduction, Fertility and Development 18:19-23, 2006; Maione et al., Molecular Reproduction and Development 50:406-409, 1998; a copy is enclosed). Therefore, the male germ cell in the paragraphs [0152]-[0156] of Rapp et al. means only spermatozoa and does not indicate spermatogonia, spermatogonial stem cells, cultured spermatogonia, cultured spermatogonial stem cells, germline stem cells, and testicular cells (except spermatozoa).

With regard to the Lin publication, Lin discloses that transfected primitive cells are injected into an embryo via yolk sac or onto the chorioallantoic membrane, see the paragraph [0137] of page 14.

> [0137]
> The method comprises transfecting primitive cells cultured in accordance with the methods disclosed herein with the DNA sequence in vitro (e.g., by electroporation or transformation with a retroviral vector), and then injecting the transfected primitive cells into an embryo, (e.g., an egg containing an embryonic bird via yolk sac or onto the chorioallantoic membrane), with the DNA sequencing being effective to cause a change in phenotype in the embryonic animal (e.g., a change in protein expression, a change in growth rate, feed efficiency, disease resistance, or a combination of all of these factors).

Thus, Lin does not disclose, teach, or suggest the technical feature of the currently amended

claim 1, referring the injection of the cultured spermatogonial cell population or testicular cell population into the most upper portion of the seminiferous tubule of a recipient.

With regard to the teachings by Li et al., in lines 33-39 of left column of page 1361, there are descriptions that the isolated cells from blastoderms of duck eggs are microinjected into subgerminal cavities of recipient eggs through a window made on the egg shells by using micropipette.

[lines 24-39 of left column of page 1361]

> Blastoderms were isolated from the freshly laid Maya duck eggs. The eggs were swabbed with 75% alcohol. Blastoderms were washed three times with PBS and dissociated with trypsin solution (Ono et al., 1994). The isolated cells were dispersed in L-15 medium at 1,000 to 2,000 cells cells/μL. The freshly laid White Leghorn chicken eggs were sterilized with 75% alcohol and randomly classified into two groups: Group 1 was exposed to 600 rad γ-irradiation, Group 2 was untreated as a control. A window, about 3 mm in diameter, was made in the egg shells. Approximately 1,000 to 2,000 cells in 1 to 2 μL of medium were microinjected into subgerminal cavities of recipient White Leghorn fertilized eggs by using a fine micropipetter with an inner diameter of about 80 μm at the tip. The window in the eggshell was sealed with sterilized film.

Accordingly, Li et al. does not also disclose, teach, or suggest the technical feature of the currently amended claims 1 and 15.

Based on our review of the differences between the present invention of claims 1 and 15 and the invention cited by the Examiner, we assert that it is clearly evident that the features of method claimed in claims 1 and 15 of the present application are not taught by Lin, Rapp et al., and Li et al. Hence, the subject matter of the present claims 1 and 15 would not have been obvious to the ordinary person skilled in the art to which the subject matter pertains.

Consequently, Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

In summary, claims 1 and 15 have been amended to specify the step of injecting spermatogonial cells or testicular cells into the most upper portion of the seminiferous tubule of the recipient. Claims 8 and 9 have been canceled. Claim 14 has been amended not to refer to canceled claims 8 and 9. Support for the amendments can be found throughout the specification, especially at page 11, lines 18 to 25. Accordingly, no new matter has been added.

As to the rejection of claims 1, 3-6, 10, and 12-15 under 35 U.S.C. § 102(b), it is clear that the Lin publication, US 2002/0076797, does not disclose the step of retrieving spermatogonial cells or testicular cells from the recipient testis.

As to the rejection of claims 1-15 under 35 U.S.C. § 103(a), it is evident that Rapp et al., Lin, and Li et al., do not disclose or suggest the technical feature of the currently amended claim 1, referring the injection of spermatogonial cells or testicular cells into the most upper portion of seminiferous tubules of the recipient embryo.

Accordingly, in view of the foregoing amendments and remarks, the Applicants respectfully request reconsideration and timely allowance of the pending claims.

A petition to extend the period for reply for one month is filed herewith, together with an authorization for charging the extension fee. If there are any other charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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